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Original Research Article

Detection of prevalence of metallo-beta lactamases in clinical isolates of imipenem resistant *Pseudomonas aeruginosa* from neonatal septicaemia cases in a tertiary hospital in Odisha, India

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a clinically troublesome gram-negative pathogen that causes both opportunistic infections and nosocomial outbreaks. Metallo beta lactamase have recently emerged as a worrisome resistance mechanism. Carbapenems had been the drug of choice for the infections caused by most penicillin- or cephalosporin-resistant gram-negative bacteria due to its broad-spectrum activity and stability to hydrolysis by most beta-lactamases. This does not hold good anymore due to rapid uprise of MBL producing strains. The current research covered 163 hospitalized cases of neonatal septicaemia from which *Pseudomonas aeruginosa* is isolated in the Paediatric Department of KIMS, Bhubaneswar. The study aimed at detecting the prevalence of metallo-beta lactamases in clinical isolates of imipenem resistant *Pseudomonas aeruginosa* from neonatal septicemia cases and to establish the antibiogram of Imipenem-resistant *P. aeruginosa* these cases.
Methods: Clinical samples obtained from suspected cases of neonatal septicemia were first cultured by conventional methods and then identification was done by VITEK-2 instrument. Metallo beta lactamase (MBL) production was done by combined disc synergy test (CDST) using imipenem and EDTA (CDST-IPM) and double disc synergy test (DDST) using IPM and EDTA (DDST-IPM).
Results: Among 1510 processed clinical specimens from cases of neonatal septicaemia; 637 (42.18%) showed positive growth of various clinically significant pathogens. Out of them in 163 (25.58%) cases *Pseudomonas* spp. was isolated. Of these, a total of 95 (58.28%) *Pseudomonas* spp. was found resistant to imipenem. All imipenem-resistant *Pseudomonas* isolates were positive for MBL by CDST imipenem-EDTA (CDST-IPM) method, whereas 89 (93.68%) were positive by DDST-IPM method, respectively. *Pseudomonas aeruginosa* was mostly isolated from endotracheal tube aspirate (57.89%) followed by pus (56.41%). Out of the 95 cases of MBL-producing *Pseudomonas*; 46 (48.42%) isolates showed the maximum susceptibility to piperacillin-tazobactam combination. All MBL-producing *Pseudomonas* isolates were resistant to ceftriaxone.
Conclusions: MBL-producing *Pseudomonas* is found to be highly prevalent in our hospital, which is one of the major causes of multidrug resistance and need regular surveillance and strict adherence to a robust antibiotic policy.

Keywords: CDST, Carbapenem resistance, DDST, Metallo-beta lactamase, Septicaemia

INTRODUCTION

The neonatal period, the first 28 days of life carries the highest risk of mortality per day than any other period during the childhood. The daily risk of mortality in the first 4 weeks of life is ~30-fold higher than the post-neonatal period, that is, from 1 month to 59 months of age. Bacterial infections are the commonest cause of

morbidity and mortality during the neonatal period. Fulminant and fatal course of infection may result from complications such as shock, disseminated intravascular coagulation and multisystem organ failure, mandating early diagnosis of this life-threatening condition for a timely treatment and a favourable outcome. In developing countries sepsis is the commonest cause of mortality responsible for 30 - 50% of the 5 million of total neonatal deaths each years.[1]

Nearly, 0.75 million neonates died in India in 2013, the highest for any country in the world.[2] The current neonatal mortality rate (NMR) is 28 per 1000 live births.[3] Early and appropriate antimicrobial therapy is one key determinant in the ultimate outcome of the patient with sepsis. This may help in reducing mortality and morbidity. The rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria is widely accepted as a major problem that has been observed over the last decade. Among the β-lactams, carbapenems are potent agents for the serious treatment of gram-negative bacterial infections. These antibiotics are well-suited to this use because of their broad-spectrum activity and resistance to hydrolysis by most β-lactamases, including the extended-spectrum. The first strain producing the Metallo-beta-lactamase (MBL) i.e., *Pseudomonas aeruginosa* was isolated in Japan in 1988.[4] Since then, the presence and spread of MBL positive strains have been observed world over, India being no exception.[5] Increased mortality rates have been documented for patients infected with MBL producing *Pseudomonas aeruginosa*, especially due to inadequate empirical therapy.

METHODS

A prospective study of one-year duration was done at Departments of Microbiology, Paediatrics and Community Medicine, KIMS, Bhubaneswar, Odisha covering a total 163 cases of neonatal septicaemia from which *Pseudomonas aeruginosa* has been isolated.

Aims and objectives of this study were to isolate *Pseudomonas aeruginosa* from various clinical samples taken from cases of suspected neonatal septicaemia, to observe the antibiotic sensitivity pattern of the *Pseudomonas* isolates and to study the prevalence of metallo-beta lactamases in clinical isolates of imipenem resistant *Pseudomonas* species.

Inclusion criteria

- All cases of neonatal septicaemia from which *Pseudomonas aeruginosa* has been isolated
- Hospitalized cases.

Exclusion criteria

- Out-door patients
- Patients with culture positive for other organisms.

Methodology

Organisms grown from various clinical samples from cases of neonatal septicaemia were first screened for the basic tests like:

- Colony characteristics on solid media (Mac Conkey Agar)
- Gram's staining of the isolated colonies
- Oxidase test.

Identification and antimicrobial susceptibility testing

The non-lactose forming, oxidase positive colonies were tested for identification and sensitivity pattern for different therapeutically relevant antibiotics by the automated VITEK-2 system (MIC break point evaluation).

Detection of metallo-beta lactamases

The isolates were tested for MBLs production by combined disc synergy test (CDST) using imipenem and EDTA (CDST-IPM) and double disc synergy test (DDST) using Imipenem (IPM) disc and Imipenem disc with EDTA (DDST-IPM).[6]

CDST-IPM

The IPM CDST was performed as described by Yong et al.[6] The test organisms were inoculated on Mueller-Hinton agar as recommended by the CLSI. A 0.5 M EDTA solution was prepared by dissolving 18.61 g of EDTA in 100 ml of distilled water and adjusting its pH 8.0 by using NaOH. The mixture was sterilized by autoclaving.

Two imipenem (10 µg) discs were placed on the surface of an agar plate at distance of 25 mm and 10 µl EDTA solution added to one of them to obtain the desired concentration of 750 µg. The zones of inhibition of imipenem and IPM discs were compared after 16 to 18 hrs of incubation in air at 37°C. In the combined disc test, if the increase in inhibition zone with the imipenem and IPM disc was found to be ≥7 µm than the imipenem alone, it was considered MBL positive.

DDST-IPM

The broth of the concerned test strains was adjusted to the McFarland 0.5 standard and used to inoculate Mueller-Hinton agar plates. Depending on the test, a 10 µg imipenem disc or a 30 µg ceftazidime disc was placed on the plate, and a blank filter paper disc was placed at a distance of 10 mm (edge to edge).[6] To the blank disc 10 µl of a 0.5 M EDTA (750 mg) solution was added. After overnight incubation, the presence of even a small synergistic inhibition zone was interpreted as MBL positive.

RESULTS

In the current study conducted at the Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, covering a very close group of study population i.e., only the hospitalized neonates; 163 non-repetitive multidrug resistant strains of *P. aeruginosa* were taken for study which were screened as oxidase positive, gram negative, motile. The further identification and sensitivity pattern was studied in fully automatic Vitek-2 instrument.

During the study period, total of 1510 non-repeating clinical specimens were processed and from them positive growth was observed in 637 (42.18%) cases and out of which 163 *Pseudomonas* spp. (25.58%) were recovered. Of the total number of 163 *Pseudomonas* isolates, a total of 95 (58%) *Pseudomonas* spp. were found resistant to imipenem and the rest 68 (42%) were sensitive to imipenem (Figure 1).

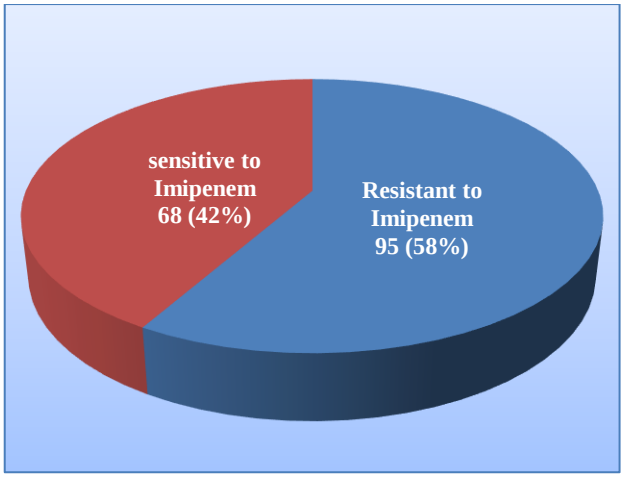

Figure 1: Imipenem sensitivity pattern of pseudomonas spp. isolated (n=163).

Table 1: Sample wise distribution of culture positive cases.

Sample	Total number	Culture positive	Percentage of positivity	*Pseudomonas aeruginosa* detected from the positives	% age of *Pseudomonas* positivity
Blood	662	311	46.97	47	15.11
Urine	473	125	26.42	31	24.8
CSF	76	1	1.31	0	0
Tracheal aspirate	185	128	69.18	46	35.93
Pus	46	39	84.78	22	56.41
Endotracheal tube aspirate	33	19	57.57	11	57.89
Others	35	14	40	6	42.85
Total	1510	637	42.18	163	25.58

Table 2: Antibiotic sensitivity pattern of *P. aeruginosa* isolates.

Antibiotics	Sensitivity shown by imipenem resistant *Pseudomonas aeruginosa* (n=95) (%)	Sensitivity shown by imipenem sensitive *Pseudomonas aeruginosa* (n=68) (%)
Ampicillin	17 (17.89)	41 (60.29)
Amoxycillin-clavulinic acid	21 (22.10)	59 (86.76)
Piperacillin-Tazobactam	46 (48.42)	63 (92.64)
Cefuroxime	3 (3.15)	41 (60.29)
Ceftriaxone	0 (0)	43 (63.23)
Cefoperazone sulbactam	11 (11.57)	57 (83.82)
Cefepime	4 (4.21)	41 (60.29)
Amikacin	21 (22.10)	63 (92.64)
Gentamicin	23 (24.21)	63 (92.64)
Ciprofloxacin	35 (36.84)	58 (85.29)
Tigecycline	10 (10.52)	63 (92.64)
Nitrofurantoin	11 (11.57)	58 (85.29)
Colistin	39 (41.05)	64 (94.11)
Trimethoprim/Sulfamethoxazole	18 (18.94)	40 (58.82)

Imipenem-resistant *Pseudomonas* spp. was most commonly isolated from endotracheal tube aspirate (57.89%), pus (56.41%) followed by other clinical samples (like catheter tip, broncho alveolar lavage etc.) (42.85 %), tracheal aspirate (35.93%), urine (24.8%), blood (15.11%) respectively (Table 1). Among the total

1510 clinical samples, 637 (42.18%) were positive and the positivity was highest among pus (84.28%) followed by tracheal aspirate (69.18%), endotracheal tube aspirate (57.57%), blood (46.97%), other clinical samples (like catheter tip, broncho alveolar lavage etc. (40%), urine (26.42%) and CSF (1.31%) respectively.

Table 3: Distribution of various methods used to detect MBL production.

MBL detection method	No. of cases (n=95)	%
Positive pattern by CDST-IPM	95	100
Positive pattern by DDST-IPM	89	93.68

Out of 95 imipenem-resistant Pseudomonas isolates, all 95 (100%) were positive for MBL by CDST-IPM method, whereas 89 (93.68%) were positive by DDST-IPM method, respectively (Table 3).

DISCUSSION

Metallo-β-lactamase-producing *Pseudomonas aeruginosa* (MPPA) is an important nosocomial pathogen that shows resistance to all β-lactam antibiotics except monobactams. The first strain producing the Metallo-beta-lactamase (MBL) i.e., *Pseudomonas aeruginosa* was isolated in Japan in 1988. Since then, the presence and spread of MBL positive strains have been observed world over, India being no exception. Increased mortality rates have been documented for patients infected with MBL producing *Pseudomonas aeruginosa*, especially due to inadequate empirical therapy.

Present study shows a quite alarming 58 % MBL positive Imipenem resistant *Pseudomonas aeruginosa* cases which is quite close to the study done by Peleg et al who have described a two year study from Alfred hospital, showing 55.8% MBL positive isolates, Doguen young et al from Korea showed 50% of MBL production in *Pseudomonas aeruginosa*.[5,13]

P. aeruginosa-producing MBL was first reported in India in 2002.[7,8] Present study covered 95 imipenem-resistant *Pseudomonas* isolates and all 95 (100%) of them were positive for MBL by CDST-IPM method and 89 (93.68%) were positive by DDST-IPM method. This is comparable to study of Sood et al (100%), Irfan et al.[10] (100%), Attal et al (88.89%), and Fam et al (87.5%).[9-12]

In the present study, imipenem-resistant *Pseudomonas aeruginosa* was most commonly isolated from endo-tracheal tube aspirate (57.89%) which is slightly on the higher side when compared with the study of Attal et al (43.7%).[11] Since there are no standard guidelines for detection of MBL, different studies have reported the use of different methods.

PCR analysis is the gold standard method for the detection of MBL production, but it is not feasible in the routine microbiology laboratory.[13] In the study of Nandi A et al (59.08%) and Dr. Khakhkhar et al 61.53% of the imipenem-resistant *Pseudomonas aeruginosa* was isolated from pus which is comparable to our finding of 56.41% positivity from pus sample.[14,15] However, in the study of Morais R et al and Sood S et al, imipenem-resistant *Pseudomonas aeruginosa* was most commonly isolated from respiratory secretions, 52.7%, and 39.13%, respectively, whereas in the present study, 57.89% of the imipenem resistant *Pseudomonas aeruginosa* was isolated from endo tracheal tube aspirate and 35.93 % from tracheal aspirate.[17,18] The isolation of this notorious organism mostly from the respiratory secretion clearly indicates its association with hospitalized patients.

A study done by Navaneeth et al from Bangalore showed that 12% isolates were resistant to carbapenems and all are MBL producers whereas in the present study 95 out of the total 163 isolates of *Pseudomonas aeruginosa* (58.28%) were found to be resistant to Imipenem and out of them all were MBL producers as determined by the phenotypic methods.[7,8]

In a study from Vellore by JesudasonIn MV et al, 75% were found to be MBL producers by EDTA disc synergy test.[16] In the present study maximum number of imipenem-resistant *Pseudomonas* isolates were susceptible to piperacillin-tazobactam combination (48.42%) followed by colistin (41.05%), ciprofloxacin (36.84 %), gentamicin (24.21 %), amikacin (22.1%), amoxicillin-clavulinic acid (22.1%), trimethoprim/sulfamethoxazole (18.94%), ampicillin (17.89%), cefoperazone-sulbactam (11.57%), tigecycline (10.52%), cefepime (4.21%) and cefuroxime (3.15%) respectively (Table 2).

All the imipenem resistant isolates were also found to be resistant to ceftriaxone. Production of MBL by pseudomonas species has tremendous consequences, since these organisms also carry other multi drug resistance genes and the only viable treatment option remains Polymyxin B which is considered as one of the potentially toxic drugs and is considered as a reserve antibiotic in hospitals following a standardized antibiotic policy.

Metallo-β-lactamase-producing *Pseudomonas aeruginosa* (MPPA) is an important nosocomial pathogen that shows resistance to all β-lactam antibiotics except monobactams. This has been reported in several countries.[18]

Carbapenems are often used as a last resort for treating serious infections attributable to multidrug-resistant gram-negative bacilli because these drugs are stable even to extended-spectrum and AmpC [beta]-lactamases. MBL production is the major cause for resistance to carbapenem group of antibiotics which are also considered to be effective drugs for treatment of infections caused by *P. aeruginosa*.[19,20]

By combined disc synergy test (CDST) using imipenem and EDTA (CDST-IPM) and double disc synergy test (DDST) using Imipenem (IPM) disc and Imipenem disc with EDTA (DDST-IPM); we can detect MBL production phenotypically in the laboratory. Routine detection of MBLs will ensure optimal patient care and timely introduction of appropriate infection control.

CONCLUSION

There is interplay of different demographic, educational, socioeconomic, biological and care-seeking factors, which are responsible for the disparities and the high burden of neonatal mortality. The country has to increase the coverage of key interventions and also improve the quality of care in health facilities on an urgent basis.

From the current study it can be inferred that Metallo beta lactamases producing Pseudomonas aeruginosa are quite higher in incidence in Kalinga Institute of Medical Sciences. This emphasizes the necessity for recognition of Metallo beta lactamase producing isolates, rigorous infection control and restricted clinical use of broad spectrum beta lactamases including carbapenems. This scenario at our hospital will definitely change because the gene responsible for MBL production is carried on mobile genetic element resulting in mutational changes in those resistant strains and converting them to sensitive ones.

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REFERENCES

1. Sarangi KK, Pattnaik D, Mishra SN, Nayak MK, Jena J. Bacteriological profile and antibiogram of blood culture isolates done by automated culture and sensitivity method in a neonatal intensive care unit in a tertiary care hospital in Odisha, India. Int J Adv Med. 2015;2:387-92.
2. Registrar General of India. Sample registration system (SRS) statistical report 2013. New Delhi; 2013.
3. Sankar MJ, Neogi SB, Sharma J, Chauhan M, Srivastava R, Prabhakar PK et al. State of newborn health in India. J Perinatol. 2016;36(Suppl 3):S3-S8.
4. Hong DJ, Bae IK, Jang IH, Jeong SH, Kang HK, Lee K. Epidemiology and characteristics of metallo-β-lactamase-producing *Pseudomonas aeruginosa*; Infect Chemother. 2015;47(2):81-97.
5. Watanabe S, Iyobe S, Inoue M, Mitsuhashi S. Transferable imepenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 1991;35:147-51.
6. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas spp*. and *Acinetobacter spp*. J Clin Microbiol. 2003;41:4623-9.
7. Peleg AY, Franklin C, Bell JM, Spelmann DW. Dissemination of the metallo-betalactamase gene blaIMP-4 among gram-negative pathogens in a clinical setting in Australia. Clinical Infectious Diseases. 2005;41:1549-56.
8. Navaneeth BV, Sridaran D, Sahay D, Belwadi MR. A preliminary study on metallo-beta-lactamase producing Pseudomonas aeruginosa in hospitalized patients. Indian J Med Res. 2002;116:264-7.
9. Sood MS. Phenotypic tests for detecting incidence of metallo-β-lactamase producing Pseudomonas aeruginosa in Jaipur. Natl J Lab Med. 2014;3:22-7.
10. Irfan S, Zafar A, Guhar D, Ahsan T, Hasan R. Metallo-beta lactamase producing clinical isolates of *Acinetobacter* species and *Pseudonomas aeruginosa* from intensive care unit patients of a tertiary care hospital. Indian J Med Microbiol. 2008;26:243-5.
11. Attal RO, Basak S, Mallick SK, Bose S. Metallo-beta-lactamase producing Pseudomonas aeruginosa: An emerging threat to clinicians. J Clin Diagn Res. 2010;4:2691-6.
12. Fam N, Diab M, Helmi H, El-Defrawy I. Phenotypic detection of metalloβ-lactamases and extended spectrumβ-lactamases among gram negative bacterial clinical isolates. Egypt J Med Microbiol. 2006;15:719-30.
13. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem EDTA disk method for differentiation of metallo beta lactamases producing clinical isolates of *Pseudomonas* sps and *Acinitobacter* sps. J Clin Microbiol. 2002;40(10):3798-801.
14. Mitra AN, Bhattacharya S, Pramanik SB, Chattopadhyay S. A study on metallo-β-lactamase producing imepenem non-susceptible multi-drug resistant Pseudomonas aeruginosa in different clinical specimens in a tertiary care hospital in Kolkata. (IOSR-JDMS). 2014;13:13-7.
15. Khakhkhar VM, Thangjam RC, Bhuva PJ, Ballal M. Detection of metallobeta-lactamase enzymes producing Pseudomonas aeruginosa isolated from various clinical samples. Natl J Integr Res Med. 2012;3:4-9.
16. Jesudason MV, Dathil AJK, Balaji V. Comparison of two methods to detect carbapenemase and metallo beta lactamase production in clinical isolates. Indian J Med res. 2004;121:780-3.
17. Luz RM. Phenotypic detection of metallo-β-lactamases in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from hospitalized patients in São Luis, State of Maranhão, Brazil. Rev Soc Bras Med Trop. 2013;46:506-9.
18. Sood S. Phenotypic Tests for detecting incidence of Metallo-B-Lactamase (Mbl) Producing *Pseudomonas aeruginosa* in Jaipur. National J Laboratory Medi. 2014;3(2):22-7

19. Navon-Venezia S, Ben-Ami R, Carmeli Y. Update on Pseudomonas aeruginosa and Acinetobacter baumannii infections in the healthcare setting. Curr Opin Infect Dis. 2005;18(4):306-13.
20. Gales AC, Menezes LC, Silbert S, Sader HS. Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM metallo-beta-lactamase. J Antimicrob Chemother. 2003;52:699-702.

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